Journal of Medical Virology 53:288–294 (1997)

Correlation Between the Detection of Viral DNA by the Polymerase Chain Reaction in Peripheral Blood Leukocytes and Serological Responses to Human Herpesvirus 6, Human Herpesvirus 7, and Cytomegalovirus in Renal Allograft Recipients

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Diagnosis of significant infections by human herpesvirus 6 (HHV6) and 7 (HHV7) in transplant patients has proved difficult because both viruses are ubiquitous and can cause persistent infections in their hosts. The significance of viral DNA detected in peripheral blood leukocytes (PBLs; DNAemia) by PCR is therefore unclear. The interpretation of serological results is complicated by the fact that both primary and secondary infections with other herpesviruses may be associated with a concurrent antibody response to HHV6. Fifty-four renal allograft recipients were studied prospectively and their serological response to HHV6, HHV7 and CMV were compared with the detection of viral DNAemia from the homologous and heterologous viruses. Serum and heparinised blood samples were collected prospectively from 54 renal allograft recipients. DNA was extracted from PBLs and tested for the presence of HHV6, HHV7 and CMV DNA by PCR. Antibodies to HHV6 and HHV7 were measured by an indirect immunofluorescence test and to CMV by an anticomplement immunofluorescence (ACIF) test. CMV IgM antibodies were detected by a commercial enzyme immunoassay. CMV and HHV7 DNAemia were each significantly associated with serological responses to the homologous virus but no such association was found for HHV6 DNAemia. However, patients with consecutively positive DNAemia to any of the viruses (including HHV6) were more likely to have a homologous serological response. Patients who had detectable CMV IgM without a concurrent rise in CMV antibodies were significantly less likely to have CMV DNAemia (odds ratio = 0.16; 95% CI 0.02–0.9). CMV IgM antibodies may be associated with HHV6 or HHV7 DNAemia (odds ratio 2.3; 95% CI 0.5–15). This serological profile may reflect a cross-reactive response to HHV6, HHV7 or other herpesviruses. CMV IgM should not be used in isolation for the diagnosis of CMV infection or disease in this group of patients. ***J. Med. Virol. 53: 288–294, 1997.***

KEY WORDS: human herpesvirus 6 (HHV6) and 7 (HHV7); cytomegalovirus (CMV); polymerase chain reaction (PCR); serological responses; peripheral blood leukocytes; DNAemia; renal allograft

INTRODUCTION

Human herpesviruses 6 (HHV6) and 7 (HHV7) are lymphotropic viruses [Salahuddin et al., 1986; Frenkel et al., 1990] distinct from each other and from other human herpesviruses [Berneman et al., 1992]. Phylogenetically both viruses are closely related to human cytomegalovirus (CMV) and are likely to be classified among the β-herpesviruses [Thomson et al., 1991; Berneman et al., 1992]. Primary infections with HHV6 and HHV7 occur during early childhood and most (>90%) of the adult population are seropositive for both viruses [Okuno et al., 1989; Clark et al., 1993]. Both viruses persist throughout adult life and are frequently shed in the saliva [Levy et al., 1990; Black et al., 1993].

HHV6 causes exanthem subitum, a benign disease of childhood [Yamanishi et al., 1988] but the association between HHV6 infection and disease in adults is less

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Accepted 9 July 1997

well defined although a mild illness with lymphadenopathy [Niederman et al., 1988] and a possible association with histiocytic necrotising lymphadenitis have been reported [Eizuru et al., 1989]. No disease is yet associated with HHV7 infection although some cases of exanthem subitum may be caused by the virus [Tanaka et al., 1994]. There are preliminary reports of HHV6 infections being associated with severe disease in immunocompromised patients [reviewed by Lusso & Gallo, 1994; Cone et al., 1994; Singh & Carrigan, 1996]. Recently, it was shown that in renal transplant recipients with CMV DNAemia, concurrent HHV7 and/or HHV6 DNAemia is associated with an increased risk of progression to CMV disease [Osman et al., 1996].

Due to their ubiquitous and persistent nature, it has been difficult to assess the role of HHV6 and HHV7 in disease. Virus isolation from peripheral blood by cocultivation techniques has been reported during primary and recurrent infections [Ward et al., 1989; Okuno et al., 1990] but may also detect virus which is reactivated from latency. Isolation techniques usually involve stimulation of peripheral blood leukocytes (PBL) by interleukin-2 which will act as a growth factor for the cells and could reactivate virus as a side effect. Similarly, viral DNA is detectable by PCR in PBL from a proportion of healthy individuals [Jarrett et al., 1990; Wilborn et al., 1995; Osman et al., 1996] and the clinical significance of virus DNA detection in PBL or tissue biopsy in disease is therefore unclear.

A presumptive serological diagnosis of HHV6 and HHV7 infection is usually made by demonstrating a rising IgG antibody titre with or without an IgM response [Fox et al., 1990]. The interpretation of such increases in titre is complicated by finding that both primary and recurrent CMV and EBV infections may be associated with a concurrent rise in HHV6 antibodies. Although cross-absorption studies have shown that these anti-HHV6 responses are at least in part specific [Irving et al., 1990; Linde et al., 1990; Ward et al., 1991], it is possible that a rising antibody titre to one virus may reflect an antigenic stimulus by another [Williams et al., 1989; Yamamoto et al., 1990]. This is of particular importance in organ transplant recipients in whom infection or reactivation with one or more herpesviruses is a common occurrence.

Fifty-four renal allograft recipients were studied prospectively and their serological response to HHV6, HHV7 and CMV was compared with the detection of viral DNA in peripheral blood leukocytes (DNAemia) from the homologous and heterologous viruses. We have focused on viral DNA in PBLs rather than virus shedding from peripheral sites (e.g. saliva or urine) because, in CMV infections, viraemia is more closely associated with clinically significant disease [Meyers et al., 1990]. Furthermore, virus shedding from peripheral sites may persist for months or years after either primary infection or reactivation with all three viruses. The objectives of this study were to examine a) whether detection of HHV6 and HHV7 DNA in PBL (DNAemia) correlated with a serological response to the homologous virus b) whether there were significant heterologous serological responses to other human β-herpesviruses and c) whether a CMV IgM response correlated with the presence of homologous and heterologous herpesvirus DNA in PBLs.

METHODS

Patients and Specimens

Fifty-four patients receiving renal allografts at the Royal Victoria Infirmary in Newcastle upon Tyne during 1991–92 were studied prospectively. Their median age was 47 years (range 4–70 years), 33 were males and 21 females. Their post transplant management has been described previously [Peiris et al., 1995]. Immunosuppression was based on cyclosporin A, with prednisolone and azathioprine being added depending on the history of previous immunological graft loss and the degree of human leukocyte antigen (HLA) A-B and DR mismatches.

Heparinised (100 units of lithium sodium) and clotted blood specimens were collected pre-transplant and at 14 day intervals for 12–30 weeks post transplant (mean duration of follow up 23 weeks).

Data on the development of CMV infection and disease in these patients and the laboratory and clinical definitions used have been previously described [Peiris et al., 1995].

Polymerase Chain Reaction (PCR)

The procedures for isolating blood leukocytes, DNA extraction and the polymerase chain reaction for CMV, HHV6 and HHV7 viruses have been previously described [Osman et al., 1996]. CMV and HHV7 DNA amplification was carried out by a "single round PCR" method while HHV6 amplification used a nested PCR. Agarose gel electrophoresis was used for detecting the amplified products for all three viruses but for HHV7 the gel results were confirmed by dot-blot hybridization using a digoxigenin-labelled oligonucleotide probe detected by a chemiluminescent reaction (Boehringer–Mannheim) according to the manufacturer's instructions.

The precautions detailed by Kwok & Higuchi [1989] to avoid PCR cross-contamination were adhered to strictly. If any of the negative controls gave a positive reaction, the whole PCR run was repeated. All the PBL extracts were also tested for β-globin DNA using PCR [Lo et al., 1989] to confirm the presence of PCR amplifiable DNA, and to exclude presence of PCR inhibitors. Viral DNA detection in PBLs (DNAemia) in a single sample was regarded as sporadic DNAemia while 3 or more consecutive positive samples was regarded as persistent DNAemia.

Serology

HHV6 and HHV7 antibodies. Sera were tested for HHV6 and HHV7 antibodies by an indirect immunofluorescence test as previously described [Salahuddin et al., 1986]. Briefly, JJHAN cells infected with the AJ strain of HHV6 [Tedder et al., 1987] and Sup-T1

TABLE I. HHV6, HHV7 and CMV Pre-Transplant Serostatus of 54 Recipients and Their Donors

Virus	Donor positive/ Recipient positive	Donor positive/ Recipient negative	Donor negative/ Recipient positive	Donor negative/ Recipient negative
HHV6	50	1	2	1
HHV7	53	1	0	0
CMV	24	12	12	6

cells infected with the DC strain of HHV7 [Clark D, Jarrett RF, unpublished results] were spotted onto 12-well teflon-coated glass slides (Hendley-Essex, UK). The slides were air dried, fixed with cold acetone and kept dry at −20°C until used. Slides similarly prepared from uninfected JJHAN and Sup-T1 cells were used as controls. Sera were diluted in PBS in doubling dilutions starting from 1/20. A volume of each dilution (15 μl) was put onto a test and control well and the slides were incubated for 30 min at 37°C in a moist chamber. After washing three times in PBS, each for 5 min, fluorescein isothiocyanate-labelled goat antihuman IgG antibody (Dako Ltd, UK) was put in each well. Following incubation for another 30 min at 37°C, the slides were washed three times in PBS as before and once in distilled water (1 min). They were then examined at a magnification of 500 times on a fluorescence microscope (Olympus, Japan) with epi-illumination and interference filters. Antibody titres were expressed as the reciprocal of the highest serum dilution yielding detectable specific intracellular fluorescence. A titre of ≥1\20 was regarded as positive.

CMV antibodies. CMV antibodies were measured by the anticomplement immunofluorescence (ACIF) test [Kettering et al., 1977]. Human CMV (strain AD169) infected and uninfected human lung fibroblasts were used to prepare slides as described above. A pool of human sera negative for CMV IgG antibodies was used as a source of complement, and fluorescein-labelled anti-human C3c (Dako Ltd, UK) was used as a conjugate. Prior titration had established the optimal dilutions for complement and conjugate to be 1:50 and 1:60 respectively. Sera were diluted as above and 15 μl of each dilution was spotted onto positive and negative slides. The slides were incubated for 30 min, washed in PBS, incubated with complement for another 30 min and washed again in PBS before a further 30 min incubation with the conjugate. After a final wash with PBS and distilled water, the slides were examined under the fluorescence microscope and antibody titres estimated as detailed above.

A serological response to the three viruses was defined as seroconversion or an increase of four-fold or more in antibody titre between two consecutive samples.

CMV IgM antibodies. Sera were tested for CMV IgM by an enzyme immunoassay (Captia CMV-M, Mercia Diagnostics). The results were graded as positive or negative according to the manufacturer's instruction.

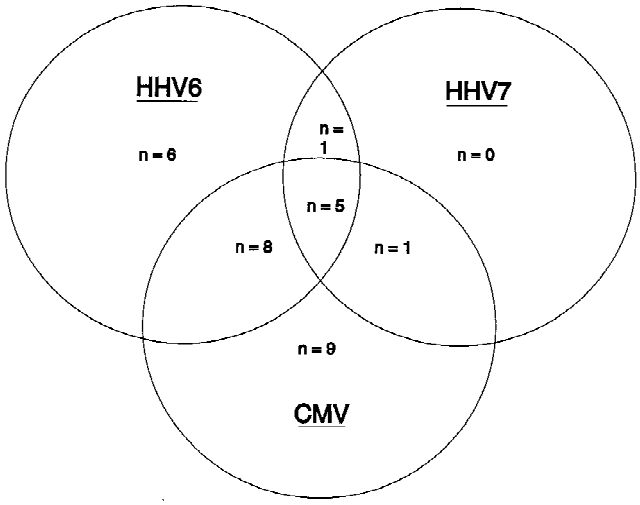

Fig. 1. Overlap in serological responses to HHV6, HHV7 and CMV.

Statistical Analysis

Fisher's exact test was used to test for associations and odds ratios were used to quantify the magnitude of association. A multiple linear logistic regression model was applied to the data. The kappa statistic (k) was used as a measure of chance-corrected proportional agreement between different assays. The strength of agreement based on the kappa statistic was graded as <0.2 = poor; 0.21–0.4 = fair; 0.41–0.6 = moderate; 0.61–0.8 = good and 0.81–1.0 = very good [Altman, 1991].

RESULTS

Pre-transplant seroprevalence of the 54 allograft recipients to HHV6, HHV7 and CMV was 96%, 98% and 67% respectively. The seroprevalence among the organ donors for these three viruses were 94%, 100% and 67% respectively. The donor-recipient serostatus patterns are shown in Table I. Both organ donor and recipient were seronegative for CMV in 6 cases, for HHV6 in one case and for HHV7 in none.

Serological responses to one or more viruses were detected in 30 (55.5%) patients during the study period. Five patients had a rising titre to all three viruses, 10 patients to two viruses and the other 15 patients had a serological response to a single virus: 6 to HHV6 and 9 to CMV (Fig. 1).

Correlation between Virus DNA in Peripheral Blood Leukocytes (DNAemia) and Serological Response to the Homologous Viruses

The correlation between DNAemia and serological response to the homologous virus is shown in Tables II and III. CMV and HHV7 DNAemia was each significantly associated with serological responses to the homologous virus but no such association was found for HHV6 DNAemia. On the basis of the kappa statistic, the relative strength of agreement between virus

TABLE II. DNAemia and Serological Response to HHV6, HHV7 and CMV in 54 Renal Transplant Patients

Serological response	No. of patients with markers for homologous virus				Fisher's exact test (*P* value)	Measure of agreement (kappa)
	DNAemia + serology	DNAemia only	Serological response only	DNAemia and serology negative		
HHV6	9	11	11	23	0.24	−0.17
HHV7	6	14	1	33	0.008	0.41
CMV:						
Rising titres (ACIF)	21	6	2	25	<0.00001	0.7
IgM response	19	8	10	17	0.014	0.33
IgM response only (no rising titre)	2	25	9	18	0.04	−0.26
Rising titres only (no IgM response)	4	23	1	26	0.35	0.02
Rising titres and/or IgM response	23	4	11	16	0.0008	0.44

TABLE III. Association between Virus DNAemia in Leukocytes and Serological Responses to Heterologous Viruses

Virus DNAemia	HHV6 IgG	HHV7 IgG	CMV rising titres	CMV IgM	CMV IgM only (no rising titres)
HHV6 DNAemia	1.7 (0.5–6.2)*	1.3 (0.2–8.8)	1.2 (0.3–4.1)	2.1 (0.6–7.8)	1.6 (0.3–7.3)
HHV7 DNAemia	1.7 (0.5–6.2)	**14.1 (1.4–670)**	1.6 (0.5–5.7)	2.1 (0.6–7.8)	1.6 (0.3–7.3)
CMV DNAemia	2.7 (0.7–9.9)	7.4 (0.8–355)	**43.7 (6.9–430)**	**4.0 (1.1–14.8)**	**0.16 (0.02–0.9)**
HHV6 or HHV7 DNAemia	1.7 (0.5–6.1)	5.3 (0.6–245)	0.9 (0.3–3.2)	2.1 (0.6–7.1)	2.3 (0.5–15.2)
HHV6, HHV7 or CMV DNAemia	3.2 (0.6–34)	undefin (0.4–infin)	**10.5 (1.2–475)**	4.1 (0.8–27)	0.6 (0.1–4.4)

*Odd ratio and 95% confidence interval. Statistically significant values are in **bold.**

DNAemia and sero-responses was good for CMV, moderate for HHV7 and very poor for HHV6.

Twenty seven patients were DNAemic for CMV during the post-transplant follow up. Twenty three of these 27 patients also had a serological response and/or an IgM antibodies to CMV: 17 of these patients having both an IgM and total antibody response (as measured by ACIF) while 2 and 4 patients respectively had an IgM or total antibody response only (Table II). Of the 27 patients with CMV DNAemia, all 13 with 3 or more consecutive viral DNAemia in their PBL specimens (persistent DNAemia) developed a serological response whereas 4/14 (29%) of the patients with sporadic DNAemia failed to do so ($P = 0.06$). The mean time for the detection of CMV DNAemia, IgM antibodies and total antibody response post-transplant was 41, 50 and 69 days respectively.

HHV6 DNAemia was detected in 20 patients and 9 of these patients had a serological response to the virus (Table II). However, overall there was no significant association between HHV6 DNAemia and the homologous serological response ($P = 0.24$). Persistent DNAemia was detected in 2 patients and both responded serologically to the virus while only 7/18 (38%) patients with sporadic DNAemia did so ($P = 0.19$). The mean time to the appearance of HHV6 DNAemia and sero-responses post-transplant was 30 and 51 days respectively.

During the study period, twenty patients had HHV7 DNAemia of whom 6 had a serological response to the virus (Table II). Overall, there was a statistically significant association ($P = 0.008$) between DNAemia and serology. Five out of 9 patients with persistent HHV7 DNAemia developed a serological response to HHV7 while only 1 of 11 patients with sporadic DNAemia did so ($P = 0.05$). The mean time to the detection of HHV7 DNAemia and serological response post-transplant was 34 and 51 days respectively.

Association between PBL Viral DNAemia and a Heterologous Serological Response

For patients with CMV, HHV6 or HHV7 DNAemia, the odds of having serological responses to homologous and heterologous viruses are shown in Table III. Patients with CMV DNAemia are 44-fold more likely to have a homologous serological response and 7-fold more likely to have an HHV7 sero-response although this association is not statistically significant.

HHV6 DNAemia was not significantly associated with serological responses to any of the other viruses studied. Nine of the 11 patients with HHV6 serological responses without detectable HHV6 DNAemia were associated with CMV or HHV7 DNAemia: 5 had CMV DNAemia, 2 had HHV7 DNAemia and 2 had both CMV and HHV7 DNAemia.

Patients with HHV7 DNAemia were 2.1-fold more likely (OR 2.1; 95% CI 0.6–7.8) to have CMV IgM response although this was not statistically significant and was much lower than that for the homologous response (OR 14; 95% CI 1.4–670).

Twenty nine patients had a detectable CMV IgM response, 11 (38%) of whom did not have rising antibody

TABLE IV. Association between Serological Responses to Heterologous Viruses

Serological response	HHV6 IgG	HHV7 IgG	CMV rising titres
HHV6 IgG	NA		
HHV7 IgG	**14.1 (1.4–669)***	NA+	
CMV rising titres	**4.5 (1.2–17)**	**10.6 (1.1–503)**	NA
CMV IgM	**6.5 (1.6–31)**	**infinity (1.4-infinity)**	**6.6 (1.7–28)**
CMV IgM only (no rising titres)	1.0 (0.2–4.5)	0.6 (0.01–6.1)	NA

*Odd ratio and 95% confidence interval. Statistically significant values are in **bold.**
+NA not applicable.

titre to CMV by ACIF. Patients with this latter serological profile were 6-fold less likely to be CMV DNAemia positive, a negative association that was statistically significant (OR 0.16; 95% CI 0.02–0.9). These patients were significantly more likely to be CMV seropositive recipients receiving kidneys from CMV seronegative donors (OR 7.4; 95% CI 1.3–41). Eight of them had either HHV6 or HHV7 DNAemia (OR. 2.3; 95% CI 0.5–15) although the latter association was not statistically significant.

Multiple linear logistic regression analysis of the data did not reveal additional information to that derived from the bivariate odds ratio analysis, and as such, these results are not presented.

Twenty seven of the patients had DNAemia detectable only to a single virus—12 to CMV, 9 to HHV6 and 6 to HHV7. Of these patients, 13 had rising antibody titres to the homologous virus (9 to CMV, 3 to HHV6 and 1 to HHV7) and 9 had rising titres to other viruses with or without response to the homologous virus. Of the 15 patients with HHV6 or HHV7 DNAemia alone, 6 had CMV IgM response, 5 of them in the absence of rising titre to CMV by ACIF.

Association between Serological Responses to Different Viruses

Seventeen patients did not have a serological response to any of the viruses studied and seven others developed only an IgM antibody to CMV. The overlap in the serological responses to the three viruses in the 30 remaining patients is shown in Fig. 1 and the associations between the serological responses to different viruses are shown in Table IV. Patients who had a serological response to one virus were significantly more likely to have response/s to one or more of the other viruses. However, the most notable associations between serological responses were those between HHV6 and HHV7 (OR 14.1) and between HHV7 and CMV (OR 10.6).

Only 15 patients had a monospecific serological response: 6 to HHV6 and 9 to CMV respectively (Fig. 1). Of the 9 with monospecific CMV responses all were associated with CMV DNAemia. Of the 6 patients with a monospecific HHV6 response, only 2 had HHV6 DNAemia and 2 others had CMV DNAemia.

DISCUSSION

Detection of CMV DNA in PBL is usually indicative of active viral infection because viral DNA is not usually detectable in the PBL of "healthy" individuals [Peiris et al., 1995]. Active infection would usually be expected to give rise to a detectable immune response although this is not invariable in immunocompromised patients. In this study, 21/27 (78%) patients with CMV DNAemia had rising IgG titres to the virus and there was a good degree of agreement between the two techniques (kappa = 0.7; Table II). While a similar number of patients with CMV DNAemia (19/27) developed IgM responses to the virus the overall agreement between DNAemia and IgM responses were less impressive (kappa = 0.32) because a number of patients with IgM responses had no detectable CMV DNAemia. It was more intriguing that the 11 patients with a CMV IgM response in the absence of a CMV IgG response were significantly (six-fold) less likely to have CMV DNAemia. Eight (73%) of these patients had either HHV6 or HHV7 DNAemia in the absence of CMV DNAemia. Although this association was not statistically significant (Table III) the trend may indicate that the IgM response to CMV detected by ELISA is more broadly cross-reactive and may reflect infection with other related viruses (e.g. HHV6 or HHV7) in addition to CMV itself. This is reminiscent of responses to some other groups of antigenically related viruses (e.g. alphaviruses) where cross-reactive IgM responses have been documented [Calisher et al., 1986]. The viral antigens in the CMV IgM ELISA assay may be different to those relevant in the ACIF assay to detect rising antibody titres to CMV. In addition, these observations may not be equally relevant to all CMV IgM ELISA assays. However, IgM ELISA assays are still in use in some laboratories for monitoring and diagnosing CMV disease in organ transplant patients. The possibility that a positive CMV IgM test may reflect a cross-reactive response to a related herpesvirus is therefor relevant and must be born in mind. Of the 29 patients with detectable CMV IgM, 11 did not have rising antibody titre to the virus and 9 of these did not have detectable CMV DNAemia. None of these 9 patients had clinically overt CMV disease. We have shown previously that the positive predictive value of CMV IgM assays for CMV disease was poor [Peiris et al., 1995]. It is now well established that alternative assays such as pp65 antigenemia [The et al., 1992] and PCR are the method of choice in surveillance and diagnosis of CMV disease in organ transplant recipients.

Unlike CMV, HHV7 DNA is detectable in PBL in a

proportion of healthy individuals who are presumed to have latent or low level persistent infection with the virus [Osman et al., 1996] making the significance of HHV7 DNAemia in PBL unclear. In this study, we showed that HHV7 PBL DNAemia and IgG responses are significantly associated with each other and this is supported by the kappa statistics (kappa = 0.41) which showed a fair agreement. Even so, only 6 of 20 (30%) patients with HHV7 DNAemia had a serological response to the virus. In contrast, there seems to be no agreement whatsoever between HHV6 DNAemia and the homologous IgG response (kappa = −0.17; OR 1.7; 95% CI 0.5–6) an observation compatible with data from other workers using PCR [Kikuta et al., 1991] or virus culture [Yoshikawa et al., 1991]. However, 3 or more consecutive PBL specimens that were positive for viral DNA are more often associated with serological responses to either HHV7 (5/9) or HHV6 (2/2) viruses. Over 40% of patients who were DNAemia positive for CMV or HHV7 have consecutively positive PBL specimens in contrast to 10% for HHV6 DNAemia [Osman et al., 1996]. The observation that HHV6 positivity in PBL is often sporadic has been noted in other studies using PCR [Appleton et al., 1996; Wilborn et al., 1994] as well as viral culture [Yoshikawa et al., 1991]. The poor overall agreement between HHV6 DNAemia and a serological response to the virus is probably due to the sporadic nature of virus activity. It seems therefore that whereas sporadic DNAemia to either HHV6 or HHV7 may be of dubious relevance, repeatedly positive PBL specimens to either virus are more likely to reflect significant virus activity. With CMV it is now recognized that two consecutive positive PCR results increase the positive predictive value of the test for clinical CMV disease in bone marrow transplant recipients and this criterion is now frequently used as a requirement for initiating pre-emptive therapy with ganciclovir [Yuen et al., 1996].

The specificity of IgG serological responses to the β-herpesviruses has been debated [Irving et al., 1990; Ward et al., 1991]. Simultaneous increases in titre to several viruses are common and it is not clear whether this represents concurrent activity of different herpesviruses or the appearance of cross-reacting antibodies. In this study only one virus was detected in PBL from 27 patients (CMV = 12; HHV6 = 9; HHV7 = 6). However, 9 of these patients had rising antibody titre to other viruses with or without the homologous immune response. We can not exclude the possibility that transient DNAemia to the relevant virus was missed because of the 14 day sampling intervals, also, peripheral virus shedding (e.g. in the saliva) may have occurred. Nevertheless the phenomenon of "original antigenic sin" may also apply to β-herpesviruses in which serological responses are directed at whichever member of a related family of viruses is first encountered during the lifespan of the patient [Francis et al., 1953].

The results of this study have implications for the diagnosis of active infections with the β-herpesviruses in immunocompromised patients. CMV IgM responses may not always reflect infection with CMV virus and may on occasion reflect activity of related β-herpesviruses. Considered overall, HHV7 DNAemia is more likely to reflect significant virus activity than HHV6 DNAemia. But when consecutive samples are positive, both HHV6 and HHV7 DNAemia are often associated with serological responses and therefore reflect virus activity. However, in future, alternative methods such as the detection of viral DNA in serum or plasma [Huang et al., 1992; Secchiero et al., 1995a] or the use of quantitative PCR [Secchiero et al., 1995b] are probably more helpful in diagnosing active HHV6 and HHV7 infections.

REFERENCES

Altman DG (1991): Practical Statistics for Medical Research. Chapman and Hall. London.

Appleton AL, Sviland L, Peiris JSM, Taylor CE, Wilkes J, Green MA, Pearson ADJ, Kelly PJ, Malcolm AJ, Proctor SJ, Hamilton PJ, Cant AJ (1995): Human herpesvirus 6 infection in bone marrow graft recipients: role in pathogenesis of graft-versus-host disease. Bone Marrow Transplantation 16:777–782.

Berneman ZN, Abalashi DV, Li G, Eger-Fletcher M, Reitz MS, Hung C-L, Brus I, Komaroff AL, Gallo RC (1992): Human herpesvirus 7 is a T-lymphotropic virus and is related to but significantly different from human herpesvirus 6 and human cytomegalovirus. Proceedings of the National Academy of Sciences USA 89:10552–10556.

Black JB, Inoue N, Kite-Powell K, Zaki S, Pellett PE (1993): Frequent isolation of human herpesvirus 7 from saliva. Virus Research 29: 91–98.

Black JB, Pellett PE (1993): Human herpesvirus 7. Reviews in Medical Virology 3:217–223.

Calisher CH, El-Kafrawi AO, Al-Deen Mahmud MI, Travassos da Rosa APA, Bartz CR, Brummer-Korvenkontio B, Haksohusodo S, Suharyono W (1986): Complex-specific immunoglobulin M antibody patterns in humans infected with alphaviruses. Journal of Clinical Microbiology 23:155–159.

Clark DA, Freeland JML, Mackie PLK, Jarrett RF, Onions DE (1993): Prevalence of antibody to human herpesvirus-7 by age. Journal of Infectious Diseases 168:251–252.

Cone RW, Wu Huang M-L, Hackman RC (1994): Human herpesvirus 6 and pneumonia. Leukemia & Lymphoma 15:235–241.

Eizuru Y, Minematsu T, Minamishima Y, Kikuchi M, Yamanishi K, Takahashi M, Kurata T (1989): Human herpesvirus 6 in lymph nodes. Lancet 1:40.

Fox JD, Ward P, Briggs M, Irving W, Stammers TG, Tedder RS (1990): Production of IgM antibody to HHV6 in reactivation and primary infection. Epidemiology and Infection 104:289–296.

Francis TJ, Davenport FM, Hennessy AV (1993): A serological recapitulation of human infection with different strains of influenza virus. Transactions of the Association of American Physicians 66: 231–239.

Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM, June CH (1990): Isolation of a new herpesvirus from human $CD4^+$ T cells. Proceeding of the National Academy of Sciences USA 87:748–752.

Huang LM, Kuo PF, Lee CY, Chen JY, Yang CS (1992): Detection of human herpesvirus-6 DNA by polymerase chain reaction in serum or plasma. Journal of Medical Virology 38:7–10.

Irving WL, Ratnamohan VM, Hueston LC, Chapman JR, Cunningham AL (1990): Dual antibody rises to cytomegalovirus and human herpesvirus type 6: Frequency of occurrence in CMV infection and evidence of genuine reactivity to both viruses. Journal of Infectious Diseases 161:910–916.

Jarrett RF, Clark DA, Josephs SF, Onions DE (1990): Detection of human herpesvirus-6 DNA in peripheral blood and saliva. Journal of Medical Virology 32:73–76.

Kettering JD, Schmidt NJ, Gallo D, Lennette EH (1977): Anticomplement immunofluorescence test for antibodies to human cytomegalovirus. Journal of Immunology 116:772–777.

Kikuta H, Itami N, Matsumoto S, Chikaraishi T, Togashi M (1991):

Frequent detection of human herpesvirus 6 DNA in peripheral blood mononuclear cells from kidney transplant patients. Journal of Infectious Diseases 163:925.

Kwok S, Higuchi R (1989): Avoiding false positives with PCR. Nature 339:237.

Levy JA, Ferro F, Greenspan D, Lennette ET (1990): Frequent isolation of HHV6 from saliva and high seroprevalence of the virus in the population. Lancet 335:1047–1050.

Linde A, Fridell E, Dahl H, Anderson J, Biberfeld P, Wahren B (1990): Effect of primary Epestien–Barr virus infection on human herpesvirus 6, cytomegalovirus and measles virus immunoglobulin G titres. Journal of Clinical Microbiology 28:211–215.

Lo Y-Md, Mehal WZ, Fleming KA (1989): In vitro amplification of hepatitis B virus sequences from liver tumour DNA and from paraffin wax embedded tissues using the polymerase chain reaction. Journal of Clinical Pathology 42:840–846.

Lusso P, Gallo RC (1994): Human herpesviruses in AIDS. Lancet 343:555–556.

Meyers JD, Ljungman P, Fisher LD (1990): Cytomegalovirus excretion as a predictor of cytomegalovirus disease after marrow transplantation: Importance of cytomegalovirus viremia. Journal of Infectious Diseases 162:273–380.

Niederman JC, Liu C-R, Kaplan MH, Brown NA (1988): Clinical and serological features of human herpesvirus 6 infection in three adults. Lancet 2:817–819.

Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K (1989): Seroepidemiology of human herpesvirus 6 in normal children and adults. Journal of Clinical Microbiology 27:651–653.

Okuno T, Higashi K, Shiraki K, Yamanishi K, Takahashi M, Kokado Y, Ishibashi M, Takahara S, Sonoda T, Tanaka K, Baba K, Yabuuchi H, Kurata T (1990): Human herpesvirus 6 infection in renal transplantation Transplantation 49:519–522.

Osman HKE, Peiris JSM, Taylor CE, Warwicker P, Jarrett RF, Madeley CR (1996): "Cytomegalovirus disease" in renal allograft recipients: Is human herpesvirus 7 a co-factor for disease progression? Journal of Medical Virology 48:295–301.

Peiris JSM, Taylor CE, Main J, Graham K, Madeley CR (1995): Diagnosis of cytomegalovirus (CMV) disease in renal allograft recipients: The role of semi-quantitative polymerase chain reaction (PCR). Nephrology Dialysis & Transplantation 10:1198–1205.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Karamarsky B, Gallo RC (1986): Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234:596–601.

Secchiero P, Carrigan DR, Asano Y, Benedetti L, Crowley RW, Komaroff AL, Gallo RC, Lusso P (1995): Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. The Journal of Infectious Diseases 171:273–280.

Secchiero P, Zella D, Crowley RW, Gallo R, Lusso P (1995): Quantitative PCR for human herpesviruses 6 and 7. Journal of Clinical Microbiology 33:2124–2129.

Singh N, Carrigan DR (1996): Human herpesvirus 6 in transplantation: An emerging pathogen. Annals of Internal Medicine 124: 1065–1071.

Tanaka K, Kondo T, Torigoe S, Okada S, Mukai T, Yamanishi K (1994): Human herpesvirus 7: Another causal agent for roseola (*exanthem subitum*). Journal of Pediatrics 125:1–5.

Tedder RS, Briggs M, Cameron CH, Honess R, Robertson D, Whittle H (1987): A novel lymphotropic herpesvirus. Lancet ii:390–392.

The TH, van Ploeg M, van den Berg AP, Vlieger AM, van der Giessen M, van Son WJ (1992): Direct detection of cytomegalovirus in peripheral blood leukocytes—A review of the antigenemia assay and polymerase chain reaction. Transplantation 54:193–189.

Thomson BJ, Martin MED, Nicholas J (1991): The molecular and cellular biology of human herpesvirus 6. Reviews in Medical Virology 1:89–99.

Ward KN, Gray JJ, Efstathiou S (1989): Brief report: Primary human herpesvirus 6 infection in a patient following liver transplantation from a seropositive donor. Journal of Medical Virology 28:69–72.

Ward KN, Sheldon MJ, Gray JJ (1991): Primary and recurrent cytomegalovirus infections have different effect on human herpesvirus 6 antibodies in immunosuppressed organ graft recipients: Absence of virus cross-reactivity and evidence of virus interaction. Journal of Medical Virology 34:258–267.

Wilborn BF, Brinkmann V, Schmidt CA, Neipel F, Gelderblom H, Siegert W (1994): Herpesvirus type 6 in patients undergoing bone marrow transplantation: Serologic features and detection by polymerase chain reaction. Blood 83:3052–3058.

Wilborn F, Schmidt CA, Lorenz F, Peng R, Gelderblom H, Huhn D, Siegert W (1995): Human herpesvirus type 7 in blood donors: detection by the polymerase chain reaction. Journal of Medical Virology 47:65–69.

Williams MV, Ablashi DV, Salahuddin SZ, Glaser R (1989): Demonstration of the human herpesvirus 6 induced DNA polymerase and DNase. Virology 173:223–230.

Yamamoto M, Black JB, Stewart JA, Lopez C, Pellett PE (1990): Identification of a nucleocapsid protein as a specific serological marker of human herpesvirus 6 infection. Journal of Clinical Microbiology 28:1957–1962.

Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T (1988): Identification of human herpesvirus 6 as a causal agent for exanthem subitum. Lancet 1065–1067.

Yoshikawa T, Suga S, Asano Y, Nakashima T, Yazaki T, Sobue R, Hirano M, Fukuda M, Kojima S, Matsuyama T (1991): Human herpesvirus-6 infection in bone marrow transplantation. Blood 78: 1381–1384.

Yoshikawa T, Suga S, Asano Y, Nakashima T, Yazaki T, Ono Y, Fujita T, Tsuauki K, Sugiyama S, Oshima S (1992): A prospective study of human herpesvirus 6 in renal transplantation. Transplantation 54:879–883.

Yuen K-Y, Lo SK-F, Chiu EK-W, Wong SS-Y, Lau Y-L, Liang R, Chan T-K, Ng M-H (1995): Monitoring of leukocyte cytomegalovirus DNA in bone marrow transplant recipients by nested PCR. Journal of Clinical Microbiology 33:2530–2534.